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Smoking as a complex but critical covariate in neurobiological studies of posttraumatic stress disorders: a review

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Abstract

As smoking rates in the general population continue to fall in response to new information and changing social values, the continued high rate of smoking among persons with psychiatric disorders has caught the attention of society at many levels: public health officials, medical and mental health care providers, and concerned family members alike. As a consequence, research studies aimed at quantifying the problem and understanding its cause have increased dramatically over the past several years. The following review first examines epidemiological studies that have revealed a bidirectional causal relationship between tobacco dependence and posttraumatic stress disorder (PTSD), one of several mental health disorders in which tobacco dependence remains prevalent and resistant to intervention. Second, we use a translational neuroscience perspective to discuss possible neurobiological mediators of the relationship between PTSD and tobacco dependence, hoping to spur further human and animal research that will elucidate pathogenetic mechanisms involved and inspire novel treatment interventions. Finally, to enable more effective clinical research in this area, we provide an overview of effective scientific methods for assessing and managing 'smoking status' as an experimental variable in clinical research studies of PTSD as well as other mental health disorders.

Keywords

smoking, nicotine, PTSD, HPA axis, fear conditioning, passive avoidance, transgenic mice, nicotinic receptors, stress, cortisol, neurosteroids

Introduction

As smoking rates in the general population continue to fall in response to new information and changing social values, the continued high rate of smoking among persons with psychiatric disorders has caught the attention of society at many levels: public health officials, medical and mental health care providers, and concerned family members alike. As a consequence, research studies aimed first at quantifying the problem and then understanding its cause have increased dramatically over the past several years.

As reviewed by Leonard *et al.* (2001), 30% of all smokers have some form of mental illness, while smokers with mental illness consume up to 45% of the cigarettes sold in the United States. Other studies have shown that 60% of persons suffering from mental illness smoke, compared to 25% of the general population. These overall figures for nicotine dependence in the mentally ill are mirrored in populations with posttraumatic stress disorder (PTSD). Shalev *et al.* (1990) first noted an increase in the rate and intensity of smoking among male military personnel with PTSD compared to those without PTSD, an observation supported by Beckham *et al.* (1997) who reported that 55% of male combat veterans with PTSD smoked, compared to 45% of combat veterans without PTSD; in addition, 48% of the combat veterans with PTSD were classified as heavy smokers compared to 28% of those without PTSD. Findings from these relatively small clinical studies appear to be confirmed by a recently reported epidemiological study in 6742 male combat veteran twins (Koenen *et al.*, 2004), wherein the prevalence of nicotine dependence was 72% in subjects with PTSD, 52% in trauma-exposed subjects without PTSD, and 40% in nontraumatized individuals. Interestingly, genetic factors accounted for 63% of the association between

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PTSD and nicotine dependence, while individually-experienced environmental factors accounted for the remainder of the association.

Comparable smoking rates have been found in women and community populations with PTSD. Acierno *et al.* (1996) reported smoking rates among traumatized women with and without PTSD to be 1.3–1.8 times that of controls. In two large community epidemiological studies, Breslau *et al.* (2003, 2004) found that trauma exposure resulting in PTSD conferred a twofold greater risk for the development of smoking dependence compared to trauma exposure not resulting in PTSD, which in turn conferred a twofold greater risk compared to no trauma exposure.

While the foregoing studies clearly demonstrate the high comorbidity between PTSD and smoking dependence, the basis for this comorbidity is not clear. As suggested by Breslau *et al.* (2003), the same vulnerabilities that lead to the development of PTSD could increase the risk for development of smoking dependence. A frequently proffered alternative hypothesis is that smoking assuages the symptoms of PTSD and serves as a means of self-medication (e.g. Beckham *et al.*, 1997). And finally, it is possible that nicotine dependence increases the risk for PTSD.

An examination of the causal relationship between nicotine dependence and PTSD has been recently completed (Koenen *et al.*, 2005) and, surprisingly, supports all three of the above hypotheses. Using survival analysis with time-dependent covariates, Koenen *et al.* (2005) found that trauma exposure and PTSD both increased the risk for nicotine dependence. In addition, nicotine dependence *doubled* the risk for subsequent development of PTSD – an important observation given the extent to which new cases of PTSD threaten to overwhelm existing mental health systems around the world (e.g. Hoge *et al.*, 2004; VanRooyen and Leaning, 2005).

The following discussion addresses possible neurobiological mediators of the comorbidity between PTSD and nicotine dependence with a particular, though not exclusive, focus on the hypothalamic–pituitary–adrenal (HPA) axis, a system that is both critically involved in the development of nicotine tolerance and affected by acute and chronic nicotine administration. In addition, numerous studies have documented variable alterations in the function of the HPA axis associated with PTSD (Yehuda, 2002; Rasmusson *et al.*, 2003), some of which appear likely to increase risk for smoking dependence. Hopefully, this discussion will spur further human and animal studies that will elucidate mechanisms involved in the pathogenesis of these disorders and inspire new approaches to their prevention and treatment. Finally, an overview of scientific methods for assessing and managing 'smoking status' as an experimental variable in clinical studies is intended to facilitate effective research in this area.

Effects of acute and chronic nicotine exposure on the HPA axis

Animal studies

Acute activation of the HPA axis by nicotine Elevation of plasma corticosterone levels by acute subcutaneous (Balfour *et al.*, 1975), intraperitoneal (Freund *et al.*, 1988), and intravenous (Grota *et al.*, 1997) injections of nicotine are reliably induced in rodents with peak levels occurring between 10 and 30 min and a return to baseline by 60–90 min. Strain differences in baseline and peak corticosterone levels, however, are evident (e.g. Grota *et al.*, 1997; Freund *et al.*, 1988). Studies primarily conducted in the rat (reviewed by Matta *et al.*, 1998) have shown that increases in corticosterone induced by nicotine are initiated by activation of nicotinic acetylcholinergic receptors (nAChRs) located on catecholamine neurons in the brainstem nucleus tractus solitarius (NTS). These catecholaminergic neurons project to and activate α_1 and α_2 adrenergic receptors located on hypothalamic paraventricular nucleus (PVN) neurons that contain corticotropin-releasing factor (CRF, also known as corticotropin-releasing hormone or CRH) (Matta *et al.*, 1990). CRF, in turn, activates adrenocorticotropin (ACTH) release from the anterior pituitary. Nicotine doses that activate ACTH release from the pituitary also activate neuropeptide Y (NPY)-containing neurons in areas A2 and C2 of the NTS; these neurons also project to the PVN where they contribute to activation of CRF synthesis and release (Matta *et al.*, 1997b) (see Fig. 1).

While the attention of research has primarily centred on interactions between nicotine and central nervous system components of the HPA axis, direct effects of nicotine on steroidogenesis in the adrenal gland also may contribute to increased cortisol levels in association with smoking. The limited research to date suggests, however, that such effects are species specific. Rubin and Warner (1975) demonstrated nicotine-induced steroidogenesis in adrenocortical cells of the cat. Krueger *et al.* (1991) showed no effect of acute or chronic nicotine on steroidogenesis in *ex vivo* adrenocortical cells of rats. Benyamina *et al.* (1987), on the other hand, showed that corticosterone and aldosterone output from *ex vivo* frog adrenal tissue is stimulated by activation of muscarinic but not nicotinic cholinergic receptors. This suggests that activation of the cholinergic nerve fibres of the splanchnic nerve, as occurs in response to neurogenic stress, could potentiate the increase in glucocorticoid secretion stimulated by nicotine effects in the central nervous system (CNS), consistent with the observed additive nature of stress and smoking on plasma cortisol levels (Pomerleau and Pomerleau, 1989). As discussed below, this might potentiate the development of nicotine dependence.

Sex-specific effects of nicotine on HPA responses also exist. Male compared to female rats show a substantially greater dose-related arginine vasopression (AVP) response to nicotine or a combination of cholinergic agonists and antagonists that mimic nicotinic receptor stimulation. Female rats show greater, dose-related ACTH and cortisol responses to nicotine (Grota *et al.*, 1997; Rhodes *et al.*, 2001). AVP potentiates the activation of ACTH by CRF and mediates novelty-induced increases in ACTH after chronic stress (Aguilera, 1994; Marti *et al.*, 1994). This suggests that nicotine-induced states of activation in chronically stressed male animals might mimic states normally triggered by exposure to novelty and promote fearful or defensive responding.

Desensitization of nicotine's effects on the HPA axis Studies in animals (e.g. Cam and Bassett, 1984) suggest that the acute effects of daily nicotine injections on plasma ACTH and cortico-

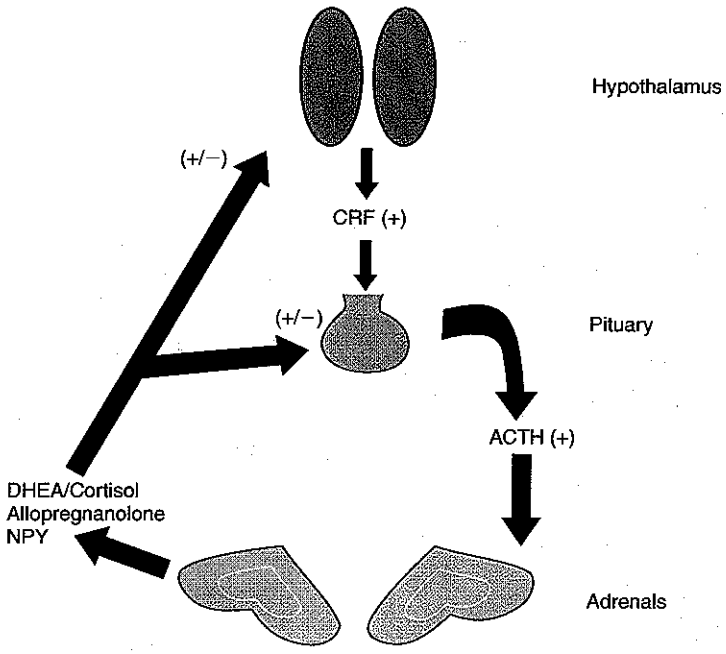

Figure 1 Hypothalamic-pituitary-axis regulation

sterone release desensitize over several days. Desensitization of the ACTH response in animals occurs more rapidly in response to more frequent intermittent delivery of nicotine, and is accompanied by an increase in the number of desensitized nAChRs. Nevertheless, norepinephrine (NE) release in the PVN is reduced by only about 50% after repeated injections of nicotine, suggesting that the more pronounced reductions in ACTH release are mediated only in part by desensitization of nAChRs in the NTS. This suggests that chronic nicotine exposure has additional effects on the HPA axis further downstream. For example, chronic nicotine exposure could potentially downregulate (a) noradrenergic receptors in the PVN, (b) CRF receptors on pituitary corticotropes, or (c) ACTH synthesis and vesicular packaging in the pituitary; in addition, factors that modulate activation of CRF receptors by CRF at the pituitary (e.g. AVP, NPY, or NE inputs) may be altered. Finally, glucocorticoid negative feedback provoked by recent activation of the HPA axis by nicotine appears to contribute modestly to reductions in ACTH responses to a second dose of nicotine (Sharp and Beyer, 1986). While not previously studied, negative feedback provided by other adrenal hormones released in response to smoking (e.g. NPY or allopregnanolone) also may contribute (Barbaccia *et al.*, 1996, 1997; Antonijevich *et al.*, 2000).

Human studies

Activation of the HPA axis by nicotine in humans In humans, activation of ACTH and cortisol release by nicotine occurs in response to relatively high doses of nicotine (reviewed by Pickworth and Fant, 1998). For example, Garcia Calzado *et al.* (1990) observed increases in ACTH in chronic smokers at 10 and 20 min after the smoking of two cigarettes containing 2.2 mg nicotine; cortisol increases were measurable only 20 min after smoking, appropriately lagging after ACTH, and correlating as one might expect, with increases in plasma levels of nicotine and cotinine, the proximal metabolic byproduct of nicotine. Coiro and Vescovi (1999) showed smoking-related increases in both plasma ACTH and cortisol levels in otherwise healthy chronic male smokers but no changes in ACTH or cortisol in chronic smokers with alcoholism who had been abstinent from alcohol for 2 and 12 weeks.

Therefore, while animal studies have demonstrated complete desensitization of HPA axis responses to nicotine after chronic nicotine exposure, human studies in medically healthy populations suggest that HPA axis responsiveness to cigarettes is maintained in chronic smokers (e.g. Garcia Calzado *et al.*, 1990; Coiro and Vescovi, 1999). This may result from resensitization of nAChRs due to the intermittent pattern of smoking adopted by many individuals.

However, it is important to point out that control populations of smoking naive individuals were not included in the studies presented. It is thus possible that HPA axis responses were at least somewhat desensitized in the individuals studied. In addition, there have been no studies of smoking effects on HPA axis functioning in populations with defined psychopathology wherein even heavier levels of smoking may occur virtually around the clock

(e.g. it is not at all uncommon for patients with severe PTSD to smoke through the evening and during the middle of the night when they cannot sleep). This is obviously a glaring deficit in the literature given the fact that rates of dependent smoking are so much higher in these groups and given that the HPA axis may play a role in nicotine-induced state changes either sought by or inadvertently experienced by these individuals. Thus, future studies should be done to determine whether such smokers retain reactivity of the HPA axis to smoking or exhibit complete desensitization.

Effects of nicotine on HPA axis reactivity to other activators in humans While HPA axis responses to smoking appear to be at least partially retained in healthy humans in response to relatively high nicotine doses, pituitary and adrenal responses to activators of the HPA axis *besides nicotine* are consistently dampened – perhaps providing an example of cross-'desensitization' (Table 1 below). Sellini *et al.* (1989a) showed that ACTH and cortisol responses to insulin-induced hypoglycaemia were markedly reduced in smokers of more than ten cigarettes per day. Kirschbaum *et al.* (1994) and Tsuda *et al.* (1996) showed decreased cortisol responses to the Trier Social Stress test in chronic smokers who recently smoked. Kirschbaum *et al.* (1994) additionally showed blunted ACTH and cortisol responses to exhaustive exercise testing and CRF challenge in smokers compared to nonsmokers. Similarly, Krishnan-Sarin *et al.* (1999) found that smokers had decreased cortisol responses to HPA axis activation by naloxone, a mu opioid antagonist that activates central noradrenergic neurons. In addition, recent smoking, compared to overnight abstinence suppressed cortisol reactivity to a mental task (Tsuda *et al.*, 1996); both of these conditions, in turn, were associated with relative blunting of cortisol responses in the smokers compared to the nonsmokers.

As noted above, work in animals suggests that decreases in reactivity of the HPA axis to environmental or endogenous stimuli may be due – but only partly – to the negative feedback provided by cortisol released in response to smoking (Sharp and Beyer, 1985). Thus it is possible that other adrenal compounds such as allopregnanolone or NPY are involved. In addition, an upregulation of glucocorticoid receptors in the pituitary, hypothalamus, or higher brain areas in response to chronic reductions in cortisol release in response to mental or physiological stress in smokers might reduce ACTH responses to CRF stimulation. This possibility is supported by the work of Sellini *et al.* (1989b) showing that nicotine-induced increases in cortisol were inhibited in smokers compared to nonsmokers after administration of 1 mg of dexamethasone the night before. And finally, it is possible that alterations in nAChRs themselves, present in pathways mediating HPA axis activation in response to natural physiological stressors, may be involved.

The blunting of cortisol responses to mental stress in smokers may be relevant to our interpretation of studies of HPA axis function in PTSD. High rates of smoking and/or the imposition of smoking abstinence in PTSD subjects participating in clinical studies may account for decreases in 24 h urinary or plasma cortisol levels observed in some PTSD populations (Rasmusson *et al.*, 2003). It is also possible that smoking-stimulated increases in plasma adrenal steroids in combination with the suppressive effects of smoking on cortisol responses to other activators of the HPA axis could contribute to the variability in the outcomes of these studies. The possible enhancement of glucocorticoid negative feedback by smoking (Sellini *et al.*, 1989b), also could account for findings of enhanced glucocorticoid negative feedback in some populations with PTSD. Thus it will be critical to characterize smoking patterns among study participants in future studies of HPA axis regulation in PTSD and mental disorders so that study findings can be accurately and confidently attributed.

Chronic smoking and baseline HPA axis-related hormone levels in humans As indicated in Table 2 below, several studies have found differences in baseline levels of HPA axis-related hormones in smokers compared to nonsmokers, but the direction of effect varies from study to study. The more variable effects of chronic nicotine exposure on the HPA axis in humans compared to laboratory animals may result from the capacity of humans to control their nicotine intake. For instance, a light, intermittent smoker exposed to lower quantities of nicotine may retain reactivity of the HPA axis in response to smoking whereas a chronic, heavy smoker may maintain a relatively high and stable plasma nicotine level and desensitize HPA axis responses to smoking. These

Table 1 Chronic smoking effects on ACTH and cortisol *responses* to physiological and mental stress

Study	Subjects	Manoeuvre	ACTH	Cortisol
Sellini *et al.* (1989a)	Males and females	Insulin-induced hypoglycaemia	Decreased (plasma)	Decreased (plasma)
Kirschbaum *et al.* (1994)	Males	CRF challenge	Decreased[a]	Decreased[a]
		Exhaustive exercise	Decreased[a]	Decreased[a]
		Trier Social Stress Test	Decreased[a] (salivary)	Decreased[a] (salivary)
Tsuda *et al.* (1996)	Males	Mental Arithmetic:		
		After overnight abstinence		Blunted
		After morning smoke		No response (salivary)
Krishnan-Sarin *et al.* (1999)	Males and females	Naloxone challenge		Decreased (plasma)

[a]Non-significant decreases in an underpowered study of 10 subjects per group; a doubling of subject number would have been necessary to achieve significance given the mean difference between the smokers and nonsmokers.

Table 2 Effects of chronic smoking on *baseline* ACTH and cortisol levels in smokers compared to non-smokers

Study	Subjects	Manoeuvre	ACTH	Cortisol	Other
Sartori *et al.* (1993) (Italy)	10 premenopausal *non-abstinent* female smokers of 2–20 cigarettes/day vs. 10 nonsmokers (all with hypertrichosis)	Variably timed plasma samples on menstrual cycle days 7, 14, and 21	Increased	Increased	Increased prolactin
Kirschbaum *et al.* (1994) (Germany)	10 male *nonabstinent* smokers vs. 10 male nonsmokers	Plasma and saliva sampled each 15 min between 9 AM and 9 PM		No difference	
Baron *et al.* (1995) (USA)	11 postmenopausal overnight abstinent female smokers of 10–25 cigarettes/day vs. 11 nonsmokers	Morning plasma		Trend for increase	Increased DHEAS and androstenedione; Trend for increased 17-OH-progesterone
Krishnan-Sarin *et al.* (1999) (USA)	5 male and 4 female overnight abstinent smokers of 20–25 cigarettes/day vs. 7 male and 4 female nonsmokers	Morning plasma		Decreased	
del Arbol *et al.* (2000) (Spain)	85 overnight abstinent male smokers vs. 22 nonsmokers	Morning plasma	Lower in heavier smokers	Increased in heavier smokers	B-endorphine increased only in light smokers (<10 cigarettes/day)

hypotheses are supported by the work of Kirschbaum *et al.* (1994) wherein: (a) the magnitude of ACTH, plasma cortisol, and salivary cortisol responses after smoking correlated positively with absolute plasma nicotine levels achieved but (b) the number of cigarettes smoked per day correlated negatively with the ACTH response. Del Arbol (2000) similarly reported that B-endorphin levels (a precursor for ACTH) were increased only in light smokers whereas ACTH levels decreased with heavier smoking.

As might be expected, given the findings presented in Table 1, baseline morning cortisol levels were lower in smokers after overnight abstinence compared to nonsmokers in a study by Krishnan-Sarin *et al.* (1999), perhaps because endogenous morning ACTH peaks were blunted in these heavy smokers (ACTH was not measured). Three studies, however, showed at least a trend for increased cortisol levels in smokers compared to nonsmokers. Cortisol levels may have been increased by recent smoking in the study by Sartori *et al.* (1993). The smokers in the studies by Baron *et al.* (1995) and del Arbol *et al.* (2000), however, were abstinent overnight. Perhaps another factor accounts for the presence of high plasma cortisol levels in these studies. The Sartori *et al.* (1993) study was conducted in a female population with hypertrichosis, a condition typically associated with increased androgen levels. The Baron *et al.* (1995) study was conducted in women with documented high androgen levels. The del Arbol *et al.* (2000) study was conducted in Hispanic males, a population in which heterozygosity for adrenal 21-hydroxylase deficiency affects up to one in four individuals (Witchel *et al.*, 1997). Heterozygosity for 21-hydroxylase deficiency, in turn, is associated both with increased androgen levels and increased cortisol reactivity to ACTH (Witchel *et al.*, 1997). We thus speculate that genetically-based increases in cortisol reactivity may be present before the onset of smoking in some individuals and contribute to the development of nicotine tolerance and high intensity, dependent smoking.

General considerations We suggest that the pattern of smoking adopted by an individual may depend on a smoker's particular psychophysiological needs. For example, some smokers may seek to suppress HPA axis activity and others to increase it in order to benefit from changes in particular adrenally-derived neuroactive compounds. Indeed, while most studies of nicotine effects on HPA axis function use cortisol as a dependent measure, cortisol is not the only adrenal hormone of relevance to mood and cognitive function (Fig. 1, also see Rupprecht, 2003). For example, adrenally-derived dehydroepiandrosteroine (DHEA), an androgenic steroid, is thought to be the primary source of DHEA and its sulfated metabolite, DHEAS, in the human brain (Compagnone and Mellon, 2000). Both DHEA and DHEAS positively modulate

N-methyl-D-aspartate (NMDA) receptor function, antagonize $GABA_A$ receptor-mediated chloride ion flux, and stimulate sigma receptors (Baulieu and Robel, 1998). Allopregnanolone, on the other hand, is an adrenal steroid with anxiolytic and anaesthetic effects due to its capacity to positively modulate brain $GABA_A$ receptors. Research in male rats has shown that allopregnanolone released from the adrenal cortex during stress contributes substantially to CNS allopregnanolone levels (Purdy, 1989). In addition, high, (0.5–2 mg/kg), but not low (0.03–0.3 mg/kg) doses of nicotine have been shown to increase brain pregnenalone, progesterone, and allopregnanolone levels in rats (Porcu *et al.*, 2003). Plasma levels of NPY increase in response to ACTH in healthy individuals (Rasmusson *et al.*, unpublished observations) and after smoking in naive subjects (Rudehill *et al.*, 1989). Increases in plasma NPY after extreme stress, in turn, have been associated with lower levels of distress and dissociation (Morgan *et al.*, 2000, 2002). In addition, all of these adrenal hormones exert feedback effects on the HPA axis. DHEA may contribute to upregulation of the HPA axis via antiglucocorticoid effects (Blauer *et al.*, 1991; Morfin and Starka, 2001), while both DHEA and DHEAS may do so via their capacity to positively modulate NMDA receptors and antagonize $GABA_A$ receptors (Baulieu and Robel, 1998). Peripheral increases in allopregnanolone (Barbaccia *et al.*, 1997) and NPY (Antonijevic *et al.*, 2000) appear to provide a dampening of ACTH and cortisol release.

Inter-individual variability in the production and release of each of these neuroactive compounds would be expected to result in differing ratios among them in the plasma or CNS after adrenal activation. Since the release of these compounds is influenced by genetic inheritance (e.g. Witchel *et al.*, 1997; Jacobs *et al.*, 1999; Kallio *et al.*, 2001) as well as experiential factors such as extreme stress exposure (e.g. Corder *et al.*, 1992; Morgan *et al.*, 2002; Sondergaard *et al.*, 2002; Morgan *et al.*, 2004), adoption of light or heavy tobacco use may be used to optimize their levels and balance. In fact, much research to date supports the idea that a balance among peripheral adrenally-derived neuroactive compounds influences mood and cognitive capacity. For example, low levels of DHEA(S) alone or in relation to cortisol have been related to depressed mood and reduced feelings of vigour and well-being in several, though not all studies (Yaffe *et al.*, 1998; Goodyer *et al.*, 1998; Cruess *et al.*, 1999; Heinz *et al.*, 1999; Michael *et al.*, 2000; Young *et al.*, 2002).

Nicotine dependence and PTSD: the chicken and the egg

Trauma exposure and PTSD facilitate nicotine dependence: a role for the HPA axis?

As reviewed by Picciotto (2003), animal studies show that similar nicotine administration paradigms result in very different outcomes with regard to nicotine self-administration, place preference, and other indicators of risk for nicotine dependence in different species of animals and even among different strains of the same species. Picciotto (2003) argues that the outcome of these studies may be influenced, in part, by baseline states of arousal and anxiety that shift the balance between activation and antagonism of central nAChRs and thereby influence the development of nicotine tolerance. We suggest that activity of the HPA axis in response to novelty or threat and subject to genetic variation mediates or at least significantly contributes to such states of arousal and anxiety – through the effects of varying proportions of neuroactive hormones released during stress. Resulting dysfunction in brain areas that modulate reward, including the frontal lobe, nucleus accumbens, and hippocampus (Chambers *et al.*, 2001) may then promote acquisition and retention of nicotine dependence.

This idea is not new. Substantial previous work indicates that the HPA axis plays a critical role in the development of nicotine self-administration and nicotine tolerance, a process leading to escalation in the dose of nicotine required to achieve desired effects (Corrigall and Coen, 1989; reviewed by Caggiula *et al.*, 1998). For example, corticosterone decreases the expression of both high and low affinity nAChRs (Pauly and Collins, 1993) and acts noncompetitively as a rapid, allosteric inhibitor of nAChR function (Ke and Lukas, 1996). Secondly, simple re-exposure of a rat to a context in which it previously received nicotine results in suppression of subsequent nicotine effects including elevations in corticosterone, locomotor suppression, and antinociception. Such 'conditioned' or learned tolerance to nicotine appears to be mediated by conditioned activation of the HPA axis (Caggiulla *et al.*, 1998) – thus, glucocorticoid or perhaps allopregnanolone- or peripheral NPY-mediated negative feedback inhibits subsequent HPA axis activation by nicotine. In addition, corticosterone is essential to the development, though not maintenance, of nicotine-sensitized locomotor responses to novelty (Johnson *et al.*, 1995). Enhancement of synaptic dopamine levels in relevant subcortical regions such as the nucleus accumbens by glucocorticoid-mediated inhibition of the extraneuronal catecholamine transporter (Grundeman *et al.*, 1998) may mediate this effect.

It thus appears that corticosterone elevations induced by any of a number of factors (novelty, stress, nicotine, conditioned contextual cues) enhances both tolerance and sensitization to various effects of nicotine. Exaggerated cortisol reactivity, as seen in *some* human populations with PTSD (reviewed by Rasmusson *et al.*, 2003), thus may facilitate acquisition of nicotine self-administration, as has been observed for amphetamine and cocaine self-administration (Piazza *et al.*, 1991; Mantsch *et al.*, 1998). In addition, PTSD patients with increased cortisol reactivity may be at greater risk for development of nicotine tolerance and dependence. Increased HPA axis activity in these subpopulations may result from a genetic predisposition (Kyllo *et al.*, 1996; Witchel *et al.*, 1997; Baghai *et al.*, 2002; Gonzalez-Gay *et al.*, 2003; Hernandez-Avila *et al.*, 2003; Smoller *et al.*, 2003; Challis *et al.*, 2004; Charmandari *et al.*, 2004; Oswald *et al.*, 2004; Slawik *et al.*, 2004; Wust *et al.*, 2004), traumatic stress exposure, perhaps at critical periods in development (Meany *et al.*, 2001; Rasmusson *et al.*, 2004), or both.

Studies examining the relationship between the HPA axis and nicotine tolerance or sensitization in animals have, however, focused almost solely on corticosterone rather than any number of

other pharmacologically potent adrenal neuroactive steroid that respond to ACTH stimulation – with one exception. Bullock *et al.* (1997) showed that the A-ring reduced metabolites of progesterone (such as allopregnanolone) reduce $^{86}Rb^{+}$ efflux from thalamic synaptosomes and nicotine-induced dopamine release from striatal synaptosomes. $^{86}Rb^{+}$ efflux under these conditions is thought to reflect the opening of nAChR ion channels. Bullock *et al.* (1997) thus suggested that these steroids act as noncompetitive inhibitors of thalamic α4/B2 nAChRs and striatal α3 nAChRs. Thus, increases in brain allopregnanolone levels stimulated by stress (Purdy *et al.*, 1991) or by high levels of nicotine administration (Porcu *et al.*, 2003) also may result in downregulation of nAChRs in the brain and contribute to the development of nicotine tolerance and dependence. Allopregnanolone may also promote nicotine tolerance through effects on nicotine metabolism. Both allopregnanolone and nicotine activate a nuclear hormone receptor called the pregnane X receptor/steroid and xenobiotic receptor (PXR/SXR or NR112) (Lamba *et al.*, 2004). Once activated, PXR induces transcription of CYP3A enzymes that promote conversion of nicotine to cotinine, the inactive primary metabolite of nicotine.

Finally, recent work has shown that DHEA induces sigma receptor-dependent reinstatement of cocaine-conditioned place preference (Romieu *et al.*, 2004). A similar mechanism may apply to reinstatement of nicotine-conditioned place preference, suggesting a role for DHEA in smoking relapse. Thus, observation of increased DHEA responses to maximal activation of the adrenal gland in premenopausal women with PTSD (Rasmusson *et al.*, 2004), increased morning plasma DHEA and DHEAS levels in Israeli combat veterans with PTSD (Spivak *et al.*, 2000), and increasing plasma DHEAS levels in refugees from Kosovo who developed PTSD (Sondergaard *et al.*, 2002) suggest that DHEA interactions with sigma receptors during stress, may contribute to refractory smoking dependence in PTSD.

Future animal studies that investigate the role of fluctuations in neuroactive steroids besides corticosterone in the development of nicotine tolerance and dependence are therefore indicated. In humans, prospective neuroendocrine studies undertaken in large populations at risk for both smoking and PTSD, such as adolescents or military personnel, may help in examining the relationship between pre-morbid neuroendocrine status, trauma exposure, PTSD and the development of refractory nicotine dependence.

Possible mechanisms underlying the pro-PTSD effects of smoking

More and more sophisticated animal studies have demonstrated extensive neurobiological effects of nicotine in the brain and on behaviour with possible relevance to the development of PTSD (Picciotto, 2003). For example, acute nicotine administration enhances passive avoidance learning (Nordberg and Bergh, 1985; Faiman *et al.*, 1991) – a phenomenon that can readily be seen to model aspects of PTSD. Gould and Wehner (1999) also have demonstrated increased *contextual* fear conditioning but normal *cued* fear conditioning in nicotine-treated animals.

Cued fear conditioning is an amygdala-mediated process wherein animals respond with fear to *discrete* cues previously paired in time with an unconditioned threatening stimulus (such as a tone that previously signalled footshock). Contextual fear conditioning is an amygdala- and hippocampus-mediated process wherein rats respond with increased fear to a *general context* previously paired with an unconditioned threatening stimulus. Ameli *et al.* (2001) has demonstrated an inverse relationship between the development of contextual and cue-based fear in humans and showed that exposure to unpredictable compared to 'signalled' threat facilitates contextual conditioning in humans. These authors further suggested that contextual fear results in more durable and unfocused anxiety than discrete 'stimulus bound' or 'cue-induced' fear and thus may increase the risk for the development of anxiety disorders such as PTSD.

Thus it is interesting that male combat veterans with PTSD compared to those without PTSD showed enhanced contextual fear conditioning in a laboratory environment (Grillon and Morgan, 1999). Since nicotine use was not quantified in this study, it is impossible to discern whether a disproportionate use of nicotine by the PTSD subjects contributed to the findings. The study also raises the possibility that a pre-morbid propensity for contextual fear conditioning might have facilitated the development of PTSD.

Nicotine's effects are not, however, confined to *facilitation* of fear conditioning. Nicotine also promotes *latent inhibition* of fear conditioning (Gould *et al.*, 2001). Latent inhibition is another hippocampus-mediated phenomenon whereby prior exposure to a context paired with safety or reward *mitigates* later association of that context with threatening unconditioned stimuli. Thus it appears that nicotine promotes hippocampus-mediated conditioning in general, facilitating associations between context and affective state whether that state is positive or negative. The direction of nicotine's effect on fear conditioning thus may depend on the novelty of the trauma context and the nature of previous experience in that context.

Finally, it is important to consider that nicotine may interfere with *extinction* of either contextual or cued fear conditioning. Rats chronically treated with nicotine did not habituate corticosterone elevations induced by placement on a high, open platform (Benwell and Balfour, 1982). Continued release of corticosterone under such conditions could increase synaptic levels of catecholamines in the frontal lobe through inhibition of catecholamine reuptake (Grundeman *et al.*, 1998). Resultant impairment in frontal lobe function and disinhibition of amygdala-mediated defensive responding could then tip the balance toward reconsolidation of the stressful experience rather than extinction (Southwick *et al.*, in press).

Fortunately, researchers have begun to elucidate specific mechanisms in brain areas such as the amygdala and prefrontal cortex (PFC) whereby nicotine administration might influence fear conditioning and extinction. For example, nicotine increases levels of both norepinephrine (Fu *et al.*, 2003) and CRF (Matta *et al.*, 1997a) in the central nucleus of the amygdala, effects that would be expected to increase fearful, defensive responding (reviewed by Southwick *et al.*, 1999 and in press). In the cortex, Lambe *et al.* (2003) found that nicotine acts at α4/B2 nAChRs to enhance the release of glutamate from thalamocortical neuronal terminals onto

pyramidal cells in Layer V of the PFC. Layer V pyramidal neurons, in turn, provide input to important subcortical structures such as the nucleus accumbens, which signals reward, and the amygdala, which mediates aversive, as well as appetitive, associative conditioning. Layer V of the PFC also receives dense input from ventral tegmental area (VTA) dopamine neurons that respond in a graded manner to escalations in aversive stimuli (Deutch and Roth, 1990; Morrow *et al.*, 1999). Stress-induced increases in dopamine release in this area, perhaps facilitated by direct nicotine effects in the VTA and prolonged by nicotine-enhanced corticosterone effects at the catecholamine transporter, may cause PFC inhibitory outputs to go 'off-line', resulting in disinhibition of amygdala-mediated defence responses (Goldstein *et al.*, 1996; Arnsten and Goldman-Rakic, 1998; Southwick *et al.*, in press).

Studies in knock-out (Picciotto *et al.*, 1995) and transgenic mouse models (King *et al.*, 2003) also show that decreased expression of high affinity α4/B2 nAChRs in PFC layer VI corticothalamic glutamatergic neurons during critical periods of development enhances passive avoidance. Thus, downregulation of these receptors in response to nicotine or stress exposure at particular stages of development might be expected to alter stress responsiveness in a direction that increases the risk for PTSD. Indeed, studies in mice suggest that perinatal exposure to nicotine (at a time that corresponds to the third trimester in humans) results in hypersensitive passive avoidance learning in adulthood (King and Picciotto, 2003).

In summary, nicotine use by humans during exposure to novel, unpredictable stress may facilitate contextual fear conditioning and promote the development or retard recovery from anxiety disorders such as PTSD. In addition, exposure of humans to stress or nicotine at critical stages of development may induce enduring effects on stress responsiveness via downregulation of nAChRs located in particular brain circuits. Therefore, it will be important for animal researchers to investigate factors that remediate, as well as mediate the effects of nicotine in these circuits. Clinical researchers should concomitantly examine the effects of smoking and mediating neurobiological variables on the development and extinction of fear conditioning in humans.

Nicotine use as self-medication in PTSD

Finally, as previously suggested (Beckham *et al.*, 1996, 2004), individuals with PTSD may learn that smoking reduces depressive or PTSD symptoms at baseline, in reaction to stressors, or in reaction to nicotine withdrawal. This possibility is supported by the work of George *et al.* (2002) that demonstrated apparent therapeutic consequences of smoking on frontal lobe function in patients with schizophrenia, though deleterious effects in healthy controls.

The means by which smoking might reduce PTSD symptoms is not clear, though animal and human studies suggest several possibilities. For example, while a lower dose of nicotine (0.045 mg/kg) can activate norepinephrine- and NPY-containing neurons in the nucleus ' tractus solitarius, higher doses (0.135 mg/kg) activate NPY- and galanin-containing neurons in the locus coeruleus, a possible source of NPY potentiation of dopamine-mediated reward in the nucleus accumbens (Josselyn and Beninger, 1993). These higher doses of nicotine also activate NPY-containing neurons located in the A1, caudoventrolateral region of the brainstem (Matta *et al.*, 1997b), an area that gives rise to noradrenergic fibres that project to basal forebrain regions such as the amygdala and septum. NPY confers place preference when microinjected into the nucleus accumbens (Josselyn and Beninger, 1993) and reduces anxiety when microinjected into the amygdala (Heilig *et al.*, 1989; Heilig, 1995). Previous work has demonstrated low baseline plasma NPY levels and blunted NPY responses during sympathetic system activation in male combat veterans with PTSD (Rasmusson *et al.*, 2000). Providing that NPY release is also blunted in the central nervous system in PTSD, high doses of nicotine may be used by persons with PTSD to elicit NPY release in brain areas that confer reward and reduce anxiety. Such high doses of nicotine would also be expected to promote nicotine tolerance and dependence through co-activation of high levels of cortisol and allopregnanolone release.

Smoking also may influence mood and PTSD symptoms through effects on neuroactive steroids. Mulchahey *et al.* (2001) observed decreased cerebrospinal fluid (CSF) testosterone levels in male veterans with PTSD; however, smokers with and without PTSD had higher testosterone levels than other subjects in their respective diagnostic groups. Unfortunately, the study did not evaluate the relationship between CSF testosterone levels and PTSD symptoms, leaving open the possibility that smoking-related increases in testosterone could have been beneficial or deleterious (Pinna *et al.*, 2005).

Heavy smoking also may help correct deficits in brain allopregnanolone levels in PTSD (Porcu *et al.*, 2003). Rasmusson *et al.* (2005) recently observed a ~50% decrease in CSF allopregnanolone levels in nonsmoking premenopausal women with PTSD studied during the follicular phase of the menstrual cycle. In these subjects, there was a very high and significant negative correlation between CSF allopregnanolone levels and re-experiencing, as well as depressive symptoms.

More intermittent smoking, on the other hand, might be used to increase plasma DHEA(S) levels. High salivary DHEAS/cortisol ratios achieved during extreme training stress in male military personnel were found to correlate negatively with dissociative symptoms (Morgan *et al.*, 2004). Higher plasma DHEAS levels also were observed in PTSD subjects without co-morbid depression, while lower levels were associated with co-morbid depression (Sondergaard *et al.*, 2002). In addition, Rasmusson *et al.* (2004) found that relative increases in the adrenal capacity for DHEA release and higher peak DHEA/cortisol levels after ACTH administration were associated with lower avoidance and hyperarousal symptoms, and better general mood, respectively, in premenopausal women with PTSD. Alternatively, nicotine may beneficially promote the degradation of DHEA in some individuals (Wang *et al.*, 2000; Lamba *et al.*, 2004). Though associated with lower avoidance and depression symptoms, high DHEA(S) levels and increased DHEA reactivity to adrenal gland activation were found to correlate with more severe sleep disturbance in two studies (Sondergaard *et al.*, 2002 and Rasmusson *et al.*, 2004).

PTSD symptoms provoked by smoking withdrawal

Individuals with a history of depression or an anxiety disorder have more severe smoking withdrawal symptoms compared to persons with a history of neither disorder (Breslau *et al.*, 1992). Interestingly, smoking withdrawal appears to mimic or worsen PTSD symptoms in individuals with PTSD (Beckham *et al.*, 1996). Thus reinstatement of smoking to control withdrawal symptoms may be more likely in individuals with PTSD, and even more so in women with PTSD. Several, though not all studies have shown women, compared to men, to have more intense withdrawal symptoms and/or a greater propensity for relapse in response to negative affect, stress, and depression (Shiffman, 1982; Svikis *et al.*, 1986; Anda *et al.*, 1990; Gritz *et al.*, 1991; Pomerleau *et al.*, 1994; Hatsukami, 1994).

It is therefore important to consider the possibility that individuals with depressive symptoms in previous smoking cessation studies may have had PTSD. First, several PTSD symptoms overlap with depressive symptoms, while others are easily overlooked unless structured clinical diagnostic evaluations for PTSD are used – procedures not typically done in smoking cessation studies. Second, Breslau *et al.* (2000) found that persons exposed to DSMIV trauma (American Psychiatric Association, 1994), which occurs in approximately 90% of the general population (Breslau *et al.*, 1998), have a three to 12-fold increased risk for development of comorbid PTSD and depression compared to depression alone. Indeed, depression comorbid with PTSD accounted for virtually all new depression cases that occurred after trauma exposure. And finally, current or past major depression comorbid with PTSD has been associated with increased cortisol levels or reactivity in women in several studies (Heim *et al.*, 2000; Rasmusson *et al.*, 2001; Lipschitz *et al.*, 2003; Young and Breslau, 2004a, b). Thus, converging evidence suggests that PTSD and depression, HPA axis dysregulation, and female gender may be inter-related mediators of nicotine dependence and refractory smoking.

Formal studies therefore need to be undertaken to ascertain effects of smoking withdrawal on symptoms of PTSD in women compared to men. Examination of neurobiological correlates of smoking and smoking withdrawal in PTSD populations – including levels of easily measured peripheral or cerebrospinal fluid neuroactive steroids or other substrates thought to affect mood and cognition – may then elucidate processes responsible for salutary effects of nicotine in PTSD and suggest clinically effective, gender-specific smoking substitutes that may ease withdrawal. For instance, recent studies in animals (Semba *et al.*, 2004) and humans (Frederick *et al.*, 1998) have noted blunted corticosterone and lower cortisol levels, respectively, after smoking cessation. However, other neuroactive steroids should be studied as well. For example, smoking each cigarette may induce small increases in plasma DHEA levels that are countered by nicotine's capacity to activate PXR and enhance DHEA metabolism. Abrupt smoking cessation may then result in acute depletion of DHEA resulting in an increase in depression and relapse. Such a process may more readily affect women, since PXR levels are higher in women (Lamba *et al.*, 2004).

Methods for the assessment of smoking status in clinical studies

Neglect of the smoking status of subjects in PTSD studies has arisen for several reasons. When the first studies of the neurobiology of PTSD were done beginning in the mid-1980s, the concept of the cigarette as 'an efficient drug delivery device that allows smokers to regulate the dose and timing of rapid nicotine delivery to the brain [and periphery]' (Gries, 1998) had not yet been developed. In fact, smoking as a self-adopted pharmacological intervention of neurobiological and clinical significance had largely escaped the notice of the psychiatric community, perhaps because so many individuals without psychiatric disorders also smoked. Then as nicotine use began to be seen as a potential 'confound' in clinical neurobiological studies, clinical researchers rapidly came to appreciate that asking PTSD patients to abstain from smoking for the purposes of participating in research was 'asking too much'. The symptoms of withdrawal from smoking in this population were understood to be so severe that subjects asked to stop smoking would either refuse to participate in research or would not comply with the prescribed period of smoking abstinence anyway. In addition, the complexity of the possible effects of nicotine began to be sensed, generating a daunting, if not overwhelming list of questions to address if smoking was to be considered in clinical studies: Is it best to study individuals just after smoking or after a period of abstinence? How can one be sure that a subject has been abstinent? How does one know whether a subject's post-cigarette nicotine level is the same as the next subject's and if it is, does it mean the same thing in regard to the dependent variable of interest? How does one match for smoking across diagnostic groups? Should one match for the number of cigarettes smoked per day, for nicotine levels, or for patterns of use? Are smokers with PTSD fundamentally different from smokers without PTSD? If so, must one study a smoking and non-smoking PTSD group and increase the number of PTSD and healthy comparison subjects recruited to deal with the resulting statistical power issues?

Fortunately, the field of tobacco use research has exploded in the past 5 years, and of fundamental importance to clinical investigators, validated methods for quantifying tobacco use now allow researchers to effectively relate levels of nicotine exposure to neurobiological outcomes of interest.

Pertinent information regarding personal history of cigarette use and/or use of smokeless tobacco products can be obtained using a structured smoking history questionnaire such as the Structured Clinical Interview for Diagnosis of DSMIV disorders (SCID) which can be administered to either psychiatrically disordered or healthy individuals (First *et al.*, 1995a, b). In addition, subjects may be administered the Fagerstrom Test for Nicotine Dependence (FTND), a modification of the Fagerstrom Tolerance Questionnaire (Heatherton *et al.*, 1991). Scores on the FTND correlate well with biochemical indices of smoking dependence (discussed below). Alternatively, and especially if time constraints make it impossible to conduct a SCID interview, a personal history of smoking can be assessed by asking about number of years smoked, types of cigarettes smoked, number and length of

quit attempts, number of cigarettes smoked on a daily basis, and age that daily smoking was initiated. The Time Line Follow Back questionnaire (TLFB; Sobell and Sobell, 1992) can also be used to obtain quantity and frequency estimates of smoking over a specified time period. Finally, it may be useful to obtain information about secondary smoke exposure by determining with whom an individual lives and whether or not that person smokes.

Biochemical measures then can be used to verify and estimate the intensity of tobacco use. One commonly used measure is breath carbon monoxide (CO). CO is a byproduct of cigarette smoking and can easily be quantified in exhaled breath using a portable precision instrument such as the Vitalograph Breath CO, from Vitalograph Inc. (Lenexa, KS). Unfortunately, while CO is a good immediate measure of cigarette use, it is not always the best one since CO levels can be increased by concurrent marijuana smoking (Biglan *et al.*, 1985) or other sources of CO in the environment such as car exhaust, indoor fireplaces, etc. (Wald *et al.*, 1981). Moreover, CO has a half-life of about 4 h and therefore is not a good indicator of cigarettes smoked more than a few hours before CO levels are determined.

Another method for documenting and quantifying cigarette use is measurement of either plasma or salivary nicotine levels since they are highly correlated (Rose *et al.*, 1993). The half-life of nicotine is only about 2 h, however, so nicotine measurements are only good indicators of recent cigarette smoking and do not reflect longer-term patterns of nicotine use. Cotinine, on the other hand, is the proximal and principal metabolite of nicotine and has a longer half-life than nicotine (18 h). In addition, cotinine has been shown to be unaffected by environmental factors (i.e. air pollutants, certain foods) other than nicotine exposure (Haley *et al.*, 1983; Haley and Hoffman, 1986). Thus, cotinine levels are thought to be the most reliable indicator of daily nicotine intake (Benowitz, 1983; Sepkovic and Haley, 1985; Benowitz and Jacob, 1994; Perez-Stable *et al.*, 1995; Haufroid and Lison, 1998; SRNT subcommittee on Biochemical Verification, 2002; Jatlow *et al.*, 2003) and provide better confirmation of self-reported smoking quantities in adult smokers (Wagenknecht *et al.*, 1992; Pokorski *et al.*, 1994; Secker-Walker *et al.*, 1997; Shaffer *et al.*, 2000; Garriti *et al.*, 2002). Moreover, cotinine levels predict withdrawal symptoms. Specifically, individuals with lower cotinine levels have been shown to have less withdrawal compared to individuals with higher cotinine levels, whereas earlier studies showed no differences in withdrawal symptoms between heavy and light smokers when smoking was characterized on the basis of cigarette consumption (Pomerleau *et al.*, 1983).

Cotinine can be measured in plasma, saliva, or urine in the laboratory. Recently, semi-quantitative urine dipsticks that measure cotinine levels in the saliva and urine also have become available. These strips measure six levels of cotinine as follows: 1 = 1–10 ng/ml, 2 = 10–20 ng/ml, 3 = 30–100 ng/ml, 4 = 200–500 ng/ml, 5 = 500–2000 ng/ml, 6 = >2000 ng/ml. The average cotinine level in smokers, normalized for cigarette consumption, is 14 ng/ml/cigarette. The average blood cotinine concentration in addicted smokers varies between 250–350 ng/ml, though a plasma cotinine level of 50–70 ng/ml is the threshold level that can readily establish and sustain addiction.

When using the above biochemical measurements, however, it is important to keep in mind that certain factors, such as race and gender, may influence their interpretation. For example, women eliminate cotinine more quickly than men (Benowitz *et al.*, 1999). In addition, Assaf *et al.* (2002) found that serum cotinine levels agree better with self-reported cigarette use in women than in men, though another study did not find gender differences in the relationship between self-reported and biochemical indices of cigarette consumption (Perez-Stable *et al.*, 1995).

Race also influences nicotine metabolism, but the limitation of relevant data to only a few ethnic groups makes effective application of this fact difficult (Benowitz *et al.*, 1999, 2002). For example, the fractional conversion of nicotine to cotinine (presumably via the p450 2A6 oxidative pathway), the clearance of cotinine by the p450 2A6 oxidation of cotinine to 3′-hydroxycotinine, and the N-glucuronidation of both nicotine and cotinine are, on average, slower in African Americans compared to whites. In addition, Chinese-Americans metabolize nicotine more slowly while concomitantly smoking fewer cigarettes. This could result in an underestimation of the time since the last cigarette was smoked in some subjects, depending on race, and contribute to misunderstandings and strain between researchers and subjects in studies requiring specified abstinence periods or misinterpretation of abstinence status in studies of smoking cessation.

Investigators conducting biobehavioural PTSD studies also should consider standardizing or obtaining information about the amount of time passed since the last cigarette was smoked since smoking withdrawal may alter cognitive performance and influence PTSD symptoms. Symptoms related to nicotine withdrawal may be measured using instruments such as the Minnesota Nicotine Withdrawal Scale, an eight-item validated scale that assesses DSM-IV symptoms of withdrawal (Hughes and Hatsukami, 1986). Craving for cigarettes, which is a significant component of nicotine withdrawal, can be measured using the above scale or a more extensive rating instrument called the Tiffany Scale for 'Urge to Smoke' (Tiffany and Drobes, 1991).

Finally, while this review is intended to encourage investigators of the neurobiology of PTSD to consider nicotine use as a factor in the design and analysis of their research studies, smoking researchers may read this review with the idea of investigating effects of HPA axis function on the development or maintenance of smoking dependence. Thus, it is important to briefly discuss basic approaches to the study of the HPA axis, neglect of which may result in the generation of data that cannot be interpreted. First, there are diurnal effects on HPA axis activity for which researchers must control (Dickstein *et al.*, 1991). Therefore, it is generally important to measure HPA axis indices at the same time of day among subjects. If this is not possible, 'time of day' may be used as a covariate or independent factor in statistical analyses of the data. This latter approach is less desirable, though, since it decreases the power of the analyses to discriminate among subject groups and may necessitate the recruitment of a larger number of subjects. In addition, the relationship of cortisol and other adrenally-derived neuroactive compounds to 'time of day' is not linear and commonly used statistical analytic methods may not be appropriate; thus, expert consultation may be necessary. Finally, several

other factors, including physical activity, mental stress, use of psychotropic or other medications, recent food consumption, and even recent casual use of alcohol may influence HPA axis function. These factors also must be assessed and accounted for to allow for meaningful interpretation of data.

Conclusion

This review has been aimed at changing the view of tobacco use in PTSD research subjects from that of a troublesome study confound to a *complex, but critical* as well as *manageable* clinical covariate. We believe that adoption of this point of view will afford a better understanding of the pathophysiology of PTSD as well as the high comorbidity between smoking dependence and PTSD. In addition, application of techniques for characterizing the smoking patterns of research participants should be extended to studies of the neurobiological basis for other anxiety disorders (Breslau and Klein, 1999; Johnson *et al.*, 2000; Broocks *et al.*, 2002; Isensee *et al.*, 2003; Schumann *et al.*, 2004; McCabe *et al.*, 2004). We hope that investigation of the relationship between nicotine dependence and PTSD or other anxiety disorders will lead to new methods of prevention and treatment of these severely debilitating and costly neuropsychiatric conditions.

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